RINGKASAN

Purifikasi dan Karakterisasi Protein Antibakteri dari Cacing Tanah

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Cacing tanah yang terdapat di Indonesia bermacam-macam famili, yaitu famili *Enchytraeidae, Moniligastridae, Octochaetidae, Glossoscolecidae, Megascolecidae* dan *Lumbricidae*. Cacing tanah yang terbanyak ditemukan di Pulau Jawa adalah cacing tanah *Pheretima javanica, Pontoscolex corethrurus* dan *Pheretima capensis*. *Pheretima javanica* termasuk dalam famili *Megascolecidae* dan mempunyai tubuh yang besar serta panjang dibanding cacing tanah yang lain (Waluyo, 1993).

Beberapa penelitian telah membuktikan adanya daya antibakteri dari protein hasil ekstraksi cacing tanah *Lumbricus rubellus* dan *Pheretima sp.* yang dapat menghambat pertumbuhan bakteri Gram negatif *Escherichia coli, Shigella dysenteriae, Staphylococcus aureus* dan *Salmonella typhi* (Affandi, 1996; Muliasari, 1996). Ju Hyun dkk. (1998) berhasil mengisolasi dan mengkarakterisasi peptida antimikroba dari cacing tanah *Lumbricus rubellus* dan disebut *lumbricin I*. *Lumbricin I* merupakan peptida antimikroba yang mengandung asam amino prolin 15 % dari total berat kering dan mempunyai berat molekul 7,231 kDa. Selain itu, Milochau dkk. (1997) juga telah mengisolasi dan mengkarakterisasi protein antibakteri dari cairan *coelomic* cacing tanah *Eisenia fetida andrei* yang mempunyai aktivitas antibakteri dan diberi nama *fetidin* dengan berat molekul 40,0 kDa dan 45,0 kDa. Selain itu cacing tanah digunakan oleh masyarakat untuk obat tipus.

Penelitian ini dilakukan dengan tujuan menguji ekstrak protein cacing tanah di Indonesia, khususnya di Pulau Jawa yang berpotensi sebagai antibakteri, dilanjutkan isolasi dan karakterisasi protein antibakteri cacing tanah. Selain itu penelitian juga ditujukan untuk menentukan konsentrasi hambatan minimum dan uji sifat kerja protein antibakteri cacing tanah terhadap bakteri uji.

Manfaat penelitian ini diharapkan dapat menghasilkan protein murni dari cacing tanah yang bersifat antibakteri, sebagai dasar untuk pengembangan penelitian lebih lanjut dalam penggandaan protein murni dengan bioteknologi. Selanjutnya diharapkan dapat dikembangkan sebagai bahan pembuatan obat alternatif penyakit tipus dan infeksi oleh bakteri lain, sehingga akan membuka wawasan baru dalam upaya pemanfaatan bahan alam secara optimal.

Penelitian ini dilakukan dengan tahapan: (1) Identifikasi cacing tanah; (2) Pembuatan ekstrak cacing tanah dalam tiga macam pelarut yaitu: 0,50mM MOPS, 0,9 NaCl, bufer fosfat; (3) Uji aktivitas ekstrak cacing tanah; (4) Purifikasi protein antibakteri menggunakan kromatografi kolom *Anion Exchanger* dan gel filtrasi serta pemurnian dengan Native-polyacrilamide gel electroforesis; (5) Penentuan konsentrasi hambatan minimal protein antibakteri; (6) Karakterisasi protein antibakteri meliputi berat molekul, kandungan asam amino, pengaruh suhu dan pH terhadap aktivitas protein antibakteri; (7) Uji sifat kerja protein antibakteri.

Hasil uji daya antibakteri menunjukkan bahwa ke tiga cacing tanah yang diteliti semua mengandung protein antibakteri. Ekstrak *Pheretima javanica* menunjukkan aktivitas lebih tinggi dibandingkan ekstrak *Pheretima capensis* dan *Pontoscolex corethrurus*, diameter zona hambatan masing-masing sebesar antara 10,0-14,0 mm; 6,5-9,0 mm; 7,0-10,0 mm. Dibanding pelarut lain bufer MOPS paling tepat untuk pelarut protein. Fraksinasi protein *Pheretima javanica* dalam pelarut MOPS dengan kromatografi DEAE dihasilkan tiga puncak kelompok protein pada pengukuran dengan spektrofotometri (λ 280). Puncak kelompok protein ketiga mempunyai aktivitas antibakteri, dan terelusi dalam kolom DEAE dengan gradien konsentrasi 0,380 M NaCl. Protein aktif difraksinasi lebih lanjut menggunakan kromatografi filtrasi dan menghasilkan dua puncak kelompok protein. Hasil analisis dengan SDS-PAGE menunjukkan bahwa puncak pertama mempunyai berat molekul 66,0 kDa-150,0 kDa dan puncak kedua mempunyai berat molekul 7,0 kDa-55,0 kDa. Hasil uji aktivitas menyatakan bahwa puncak kedua mempunyai aktivitas antibakteri. Puncak protein kedua kemudian dimurnikan dengan Native-PAGE dan dihasilkan tujuh macam pita protein. Hasil uji aktivitas terhadap masing-masing pita menunjukan bahwa pita protein no.6 mempunyai aktivitas antibakteri. Protein tersebut mempunyai konsentrasi hambatan minimum terhadap bakteri Gram negatif: *Salmonella typhi, Shigella dysenteriae, Escherichia coli*, berturut-turut sebesar 40,0 μg/mL, 40,0 μg/mL, 20,0 μg/mL, dan Gram positif: *Psedomonas aeruginosa, Bacillus subtilis* pada konsentrasi 20,0 μg/mL, 10,0 μg/mL. Hasil karakterisasi berat molekul dengan SDS-PAGE menunjukkan bahwa pita protein no.6 (aktif) mengandung dua macam pita protein dengan berat molekul 31,0 kDa dan 34,0 kDa. Hasil analisis asam amino protein no.6 mengandung hidroksiprolin (19,04%). Uji aktivitas antibakteri masih mempunyai aktivitas sampai suhu 75°C dan mempunyai pH optimum 7. Protein antibakteri *Pheretima javanica* mempunyai sifat kerja antibakteri sebagai bakteriostatik.

Hasil penelitian ini diharapkan dapat menjadi dasar bagi penelitian lanjutan, terkait dengan protein antibakteri dari cacing tanah. Oleh karena itu disarankan untuk penelitian lebih lanjut dalam melakukan kloning DNA dari protein antibakteri *Pheretima javanica* dan penerapan obat alternatif.

SUMMARY

Purification and Characterization of Antibacterial Protein from Earthworms

Joko Waluyo

There are many kind of earthworm family in Indonesia i.e.: *Enchytraeidae, Moniligastridae, Octochaetidae, Glossoscolecidae, Megascolecidae, and Lumbricidae*. The Species of earthworm commonly found in Java are *Pheretima javanica, Pontoscolex corethrurus,* and *Pheretima capensis*. *Pheretima javanica* is the member of *Megascolecidae* family. They have a big and long body compared to the others (Waluyo, 1994).

Several research have proved that there are antibacterial activities from earthworm extract of *Lumbricus rubellus* and *Pheretima sp.;* which can inhibit the growth of Gram negative bacteria, such as *Escherichia coli, Shigella dysenteriae, Stapilococcus aureus* and *Salmonella typhi* (Affandi, 1996 and Muliasari, 1996).

Ju Hyun, et. al (1998) succeeds to isolate and characterize antimicrobial of *Lumbricus rubellus* extract and it was called *lumbricin* 1. It is contains amino acid proline about 15% (w/dry weight) and has molecular weight 7.231 kDa. Milochau et. al (1997) isolated and characterized antibacterial protein from coelomic liquid of *Eisenia fetida adrei* that has an antibacterial activity. It is called *fetidin*; which has molecular weight 40,0 kDa and 45,0 kDa.

This research has been conducted to prove that the protein extract from three species of earthworms have an antibacterial potencies. Isolation and characterization of the antibacterial protein and also their Minimum Inhibition Concentration have been performed.

The aim of this research is to find out a pure protein from earthworms; which has antibacterial activities. Results of research are expected produced novel issues in term of multiply the protein by genetic engineering. On the other hand, it can be developed as the basic materials of alternative medicine for typhoid and other infection caused by bacteria. Therefore, it will contribute a new concept for utilization of natural product.

The research has been conducted in several stages, namely (1) Earthworm determination, (2) Extraction earthworm by using three kinds of solvents, (3) Antibacterial activity test of earthworm extract by agar diffusion method, (4) Purification of antibacterial protein by using DEAE chromatography (anion exchanger), filtration column chromatography, and Native-PAGE, (5) Determination of Minimum Inhibition Concentration of antibacterial protein, (6) Characterization of antibacterial protein including determination of molecular weight, amino acid content, effect of temperature and optimum pH, and (7) The tests of antibacterial protein activity.

The results showed that three species of earthworms contained antibacterial protein. The activity of antibacterial extract of *Pheretima javanica* in MOPS solvent was higher than extract of *Pheretima capensis* and

Pontoscolex corethrurus, inhibition zone diameter each of among 10,0-14,0 mm, 6,5-9,0 mm, 7,0-10,0 mm. Compared to other solvent of MOPS buffer most precise for the solvent of protein. Then the extract of *Pheretima javanica* with MOPS solvent was fractionated by using DEAE chromatography (anion exchanger) produced three peaks of protein group; the last peak need 0,380 M NaCl concentration gradient. One of the fractions has antibacterial activity; by using filtration chromatography produced two peaks. The first peak had a molecular weight 66,0 kDa-150,0 kDa, and the second peak had a molecular weight 7,0 kDa-55,0 kDa, after tested the second peak had antibacterial activities. Further purification that carried out by cutting the active fraction of second protein peak by using Native-PAGE produced seven bands. The sixth band has antibacterial activity. The Minimum Inhibition Concentration of Gram negative bacteria *Salmonella typhi, Shigella dysenteriae, Escherichia coli* and Gram positive bacteria *Pseudomonas aeruginosa, Bacillus subtilis* was 40,0 μg/mL, 40,0 μg/mL, 20,0 μg/mL, 20,0 μg/mL and 10,0 μg/mL respectively. The sixth protein band had characteristic in molecular weight 31,0 kDa and 34,0 kDa. It contains 19.04% hydroxyproline, and has showed heat resistance at 75^0 C. The protein active of *Pheretima javanica* is a bacteriostatic.

The result of this research expected to be useful for further research in relation to antibacterial protein from earthworms. Therefore, it is recommended to conduct further research on cloning and applying alternative drug.

ABSTRACT

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This research has been conducted to prove that the protein extract from three species of earthworms have an antibacterial potencies. Isolation and characterization of the antibacterial protein and the Minimum Inhibition Concentration have been done.

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Key words: Earthworms *Pheretima javanica, Pontoscolex corethrusus, Pheretima capensis,* antibacterial protein, *Salmonella typhi, Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa, Bacillus subtilis.*